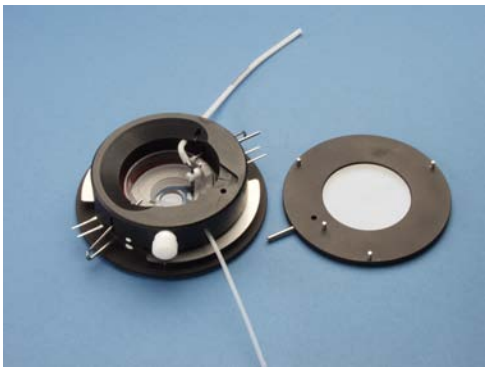
Warner Instruments, Inc.

Micro-Incubation Chamber For 35 mm Cell Culture Dishes

Model DH-35i



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The **DH-35i** Micro-Incubation Chamber from Warner Instruments is designed to provide complete environmental control over 35 mm culture dishes while maintaining the ability to make both short- and long-term imaging studies. This versatile system readily accommodates culture dishes from several manufacturers and will adapt to any microscope stage supported by Warner Instruments.

Features of the **DH-35i** include:

- ✓ Designed for glass-bottomed 35 mm cell culture dishes
- ✓ Permits imaging, temperature, and gas atmosphere control
- ✓ Adapts to Willco Wells, and Corning and Falcon dishes
- ✓ Unique dish clamps permit either open or closed use
- ✓ Works with **RC-37** family of Cell Culture Dish Perfusion Chamber Inserts

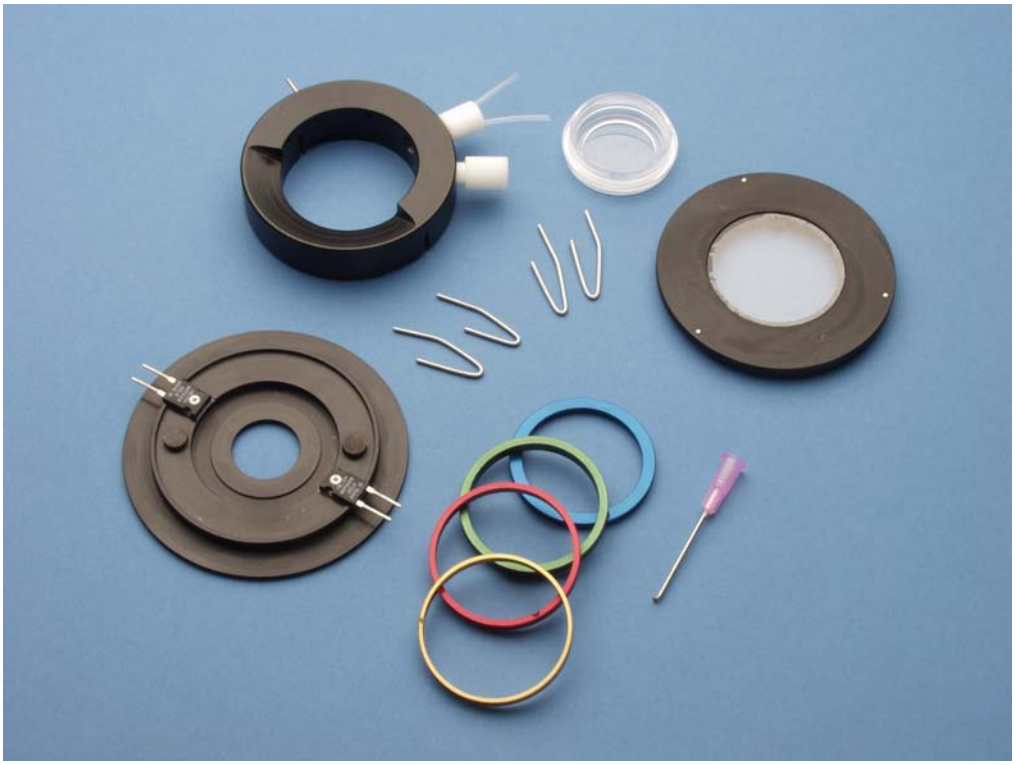
THIS EQUIPMENT IS NOT DESIGNED NOR INTENDED
FOR USE ON HUMAN SUBJECTS

INTRODUCTION

The introduction of affordable 35 mm cell culture dishes incorporating high-quality glass bottoms has extended their use into areas such as confocal microscopy, fluorescence microscopy and high resolution image analysis. The **DH-35i** Culture Dish Incubator is designed to facilitate the imaging of live cells in a cell culture dish using either an open or closed dish format.

The open dish format readily accommodates the use of microelectrodes or rapid throughput tools (such as the **RC-37** Cell Culture Dish Perfusion Chamber) while the closed dish format can be used to perform long-term studies or to maintain pH, [CO_2], or other environmental factors. Temperature control is available in both open and closed formats

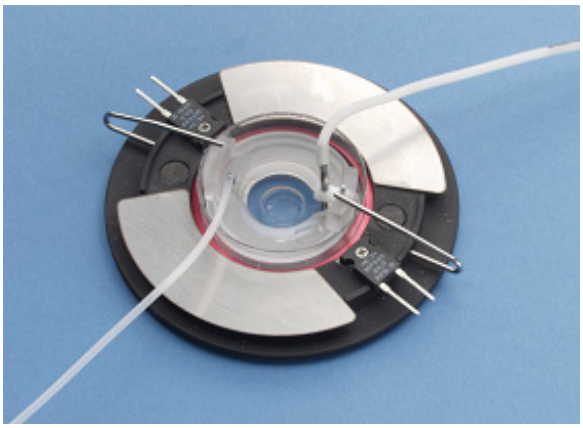
DH-35i incubators are designed to work with Willco Wells, Corning or Falcon 35 mm glass bottom cell culture dishes. The incubator is machined in three separate interlocking sections and metal adapters are supplied to insure a secure fit for supported cell culture dishes.



COMPONENTS

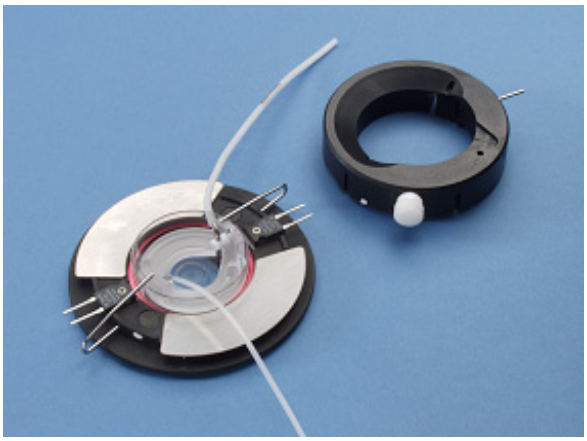
The completed **DH-35i** assembly is comprised of a bottom, middle and top section, along with culture dish adapter rings and an optional **RC-37** chamber insert. The completed assembly can be mounted onto a microscope using an appropriate microscope stage adapter and thermal control is provided using a **CC-28** cable and temperature controller.

Bottom Section



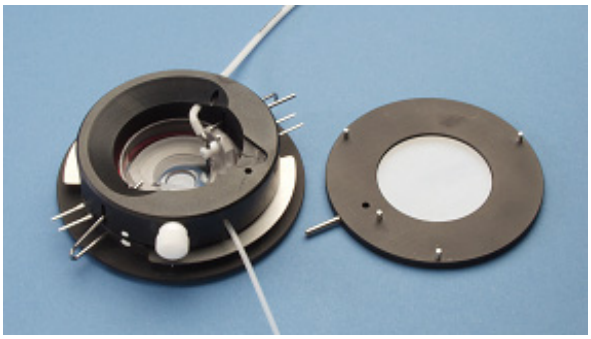
The Bottom Section positions the cell culture dish using one of four adapter rings (*see* table on next page). The bottom section also includes two resistive heating elements, a 19 mm diameter aperture, two magnetic posts for attachment of the Middle Section component, two holes for the special dish clamps, and a single hole for a attachment of a control thermistor (e.g. via Warner's **CC-28** cable). The periphery of the bottom section is framed with stainless steel plates for mounting of magnetic holders when the **DH-35i** is used on the *open configuration*.

Middle Section



The Middle Section attaches to the Bottom Section via two magnetic points mounted on the Bottom Section. The Middle Section has slots around its circumference to allow access for cabling and tubing elements.

Two recessed magnetic anchors couple directly with the Bottom Section. Several slots are provided for access of perfusate inlet and outlet lines, gas inlet and outlet lines, and a hole for placement of a bath thermistor (e.g. via Warner's **CC-28** cable).



The small chrome tube shown in the picture above can be used to supply a slight vacuum to the chamber which will keep the top cover clear of condensation while the **DH-35i** is in use.

Top Section

The Top Section rests on top of the Middle Section and forms an enclosed micro-

incubation system. Alignment is achieved via is via three metal posts and an alignment pin. The top section holds a 40 mm cover glass via a recessed annular lip and the cover glass is secured using a rubber o-ring. When in place, the cover glass seals a 36 mm widow useful for illumination purposes.

Accessories

1. Four colored metal adapter rings are included to insure a close fit between the Bottom Section and the cell culture dish. Rings are tapered for a precise fit and should be mounted with the *notch facing down*. A special tool (i.e. a modified 18G hypodermic needle) is provided to assist in removing adapter rings from the Bottom Section. Color codes for the different adapter rings are shown below.

Adapter ring color codes		
Blue	Willco Wells:	Models D3512 and D3522
Red	Falcon:	37 mm
Gold	Falcon:	38 mm
Green	Corning:	25000 series

2. Special clamps (two sets) are provided to hold cell culture dishes in place in the Bottom Section. (These clamps are shown in use in the top photo on page 5.) Clamping insures good thermal contact between the culture dish and the heated surface which helps to maintain tight temperature control.

SETUP

General Comments

The DH-35i is assembled in stages from the bottom up.

The Bottom Section may be used alone for open format applications while additional assembly of the Middle and Top Sections are required for closed format applications.

The Middle Section contains several ports allowing gas line, perfusion line, electrode and thermistor access. While the Middle Section has been designed for closed format applications, it can also be used as a multi-port holder for open format applications.

We recommend the use of the **RC-37** Culture Dish Perfusion Insert when using the **DH-35i** in closed format.

Open Format

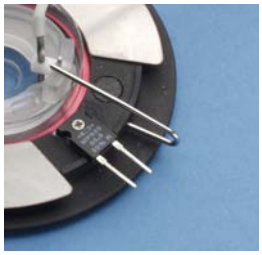
Open Format Setup without Perfusion

1. Begin by selecting the appropriate adapter ring for your cell culture dish. (*See* adapter guide above.)

2. Place the adapter ring, notch side down, on a firm surface and insert the cell culture dish into the ring by gently pushing it into place.

   NOTE: Care must be taken to evenly insert the dish into the ring so that the dish seats properly within the Bottom Section.

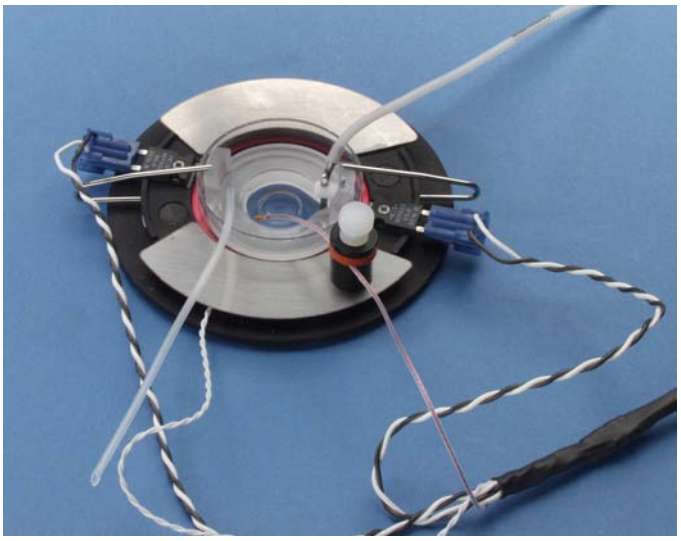
Clamping



The dish/ring assembly is placed into the recess in the Bottom Section and clamped into place. Cell culture dish clamps (2 ea) are provided and are placed into two small holes located on either side of the Bottom Section. These holes can be found directly between the magnet and heating resister, and can be located by a red colored dot on top of the rim on the Bottom Section. Clamps are placed on both sides of the dish. (*See* the figure to the right for clamp positioning.)

3. While holding down the dish with one hand, slide the clamps into position with the other hand until they secure the culture dish into place. Verify that the dish is seated completely flat.

4. If heating with Warner's **TC-324B** or **TC-344B** Temperature Controllers, slip the white colored T1 feedback thermister on the **CC-28** cable into the milled hole in the side of the Bottom Section. A small amount of mineral oil can facilitate thermal contact between the T1 feedback thermister and the metal of the Bottom Section. Slip the blued colored leads over the two resistive heating elements and then plug the **CC-28** cable into the temperature controller.



   Consult the **TC-324B/TC-344B** user's manual for methods of controlling the temperature of the clamped culture dish.

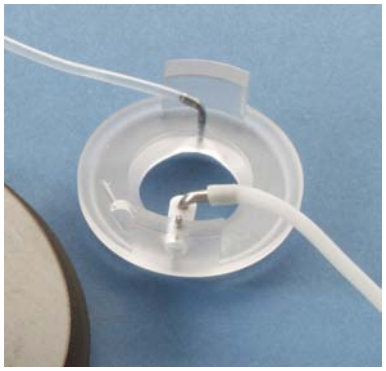
5. If imaging, place the fully assembled **DH-35i** into your microscope's **Series 20 Stage Adapter** and mount onto the microscope.

Open Format Setup with Perfusion

1. Perform steps 1 through 3 described in the ***Open Format Setup without Perfusion*** portion of this manual. (*See* page 6.)

2. Align the Bottom Section (with the already clamped dish in place) so that the white dot on the side of the Bottom Section faces towards the left (i.e. roughly 9:00). The direction of the clamps holding the dish should be horizontal, from left to right.

Placing and Clamping the RC-37



RC-37 perfusion inserts (e.g. **RC-37F, RC-37W**) have both a perfusate inlet tubing port and a suction tubing port (with a white Teflon height adjusting component). These ports are each guarded by raised sections of the insert itself.

3. With the perfusion inlet tubing port directed towards you (i.e. roughly 6:00), slip the **RC-37** between the clips and into the clamped dish. The non-raised sections of the **RC-37** should easily slip under the clamps holding the dish. When properly installed, the white Teflon height adjusting component should be near the top of the clamped 35 mm dish (i.e. roughly 12:00).
4. Rotate the **RC-37** *clockwise* until the raised sections of the insert bump up against the clamps. The suction tubing port (with its white Teflon component) will be towards the right.
5. While holding the **RC-37** with your left hand, further rotate the insert clockwise while simultaneously lifting the right side clamp (use the right hand) onto the raised section which guards the suction tubing port.
6. Next, hold down the **RC-37** with the right hand and place the left side clamp on top of the raised section which guards the inlet tubing port.

   NOTE: It is important to always manually apply downward pressure on the **RC-37** until both clamps are securely in place.
7. Center the diamond-shaped chamber of the RC-37 insert on the window of the 35 mm cell culture dish and adjust the height of the suction tubing port via its white Teflon component.
8. If heating, perform step 4 described in the ***Open Format Setup without Perfusion*** portion of this manual (*see* page 7).

Closed Format

Closed Format Setup without Perfusion

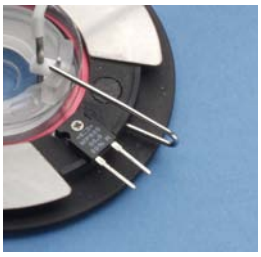
1. Begin by selecting the appropriate adapter ring for your cell culture dish. (*See* adapter guide above.)
2. Place the adapter ring, notch side down, on a firm surface and insert the cell culture dish into the ring by gently pushing it into place.

   NOTE: Care must be taken to evenly insert the dish into the ring so that the dish seats properly within the Bottom Section.

Clamping

The dish/ring assembly is placed into the recess in the Bottom Section and clamped into place. Cell culture dish clamps (2 ea) are provided and are placed into two small holes

located on either side of the Bottom Section. These holes can be found directly between the magnet and heating resister, and can be located by a red colored dot on top of the rim on the Bottom Section. Clamps are placed on both sides of the dish. (*See* the figure to the right for clamp positioning.)



3. While holding down the dish with one hand, slide the clamps into position with the other hand until they secure the culture dish into place. Verify that the dish is seated completely flat.
4. Place the Middle Section of the **DH-35i** on top of the Bottom Section so that the two white dots on the side are aligned. There are two long narrow slots for both clamps and two short wide slots for both resisters.
5. Place the Top Section of the **DH-35i** on top of the Middle Section. There is an aligning hole on the top of the Middle Section and a matching pin on the bottom of the Top Section to facilitate proper alignment. There are also three additional pins on the bottom of the Top Section which fit around the outside edges of the Middle Section.
6. If heating with Warner's **TC-324B** or **TC-344B** Temperature Controllers, slip the white colored T1 feedback thermistor on the **CC-28** cable into the milled hole in the side of the Bottom Section. A small amount of mineral oil can facilitate thermal contact between the T1 feedback thermistor and the metal of the Bottom Section. Slip the blued colored leads over the two resistive heating elements and then plug the **CC-28** cable into the temperature controller.

   Consult the **TC-324B/TC-344B** user's manual for methods of controlling the temperature of the clamped culture dish.
7. If imaging, place the fully assembled **DH-35i** into your microscope's **Series 20 Stage Adapter** and mount onto the microscope.

Closed Format Setup with Perfusion

1. Perform steps 1 through 3 described above in the ***Closed Format Setup without Perfusion*** portion of this manual. (*See* page 8.)
2. Attach C-Flex tubing to the output port and PE-50 tubing to the input port on your **RC-37** Insert.
3. Align the assembled **DH-35i** so that the white dot on the side of the Bottom Section faces towards your left (i.e. roughly 9:00).
4. While holding the Middle Section of the **DH-35i** with *its* white dot also facing towards your left, slip a portion of the **RC-37** suction tubing through its matching slot on the right side of the Middle Section (i.e. roughly at 2:00). Give yourself excess flexible tubing inside the Middle Section at this time.

5. Place the narrow slot and wide slot on the right side of the Middle Section on top of the matching right-side clamp and resister respectively (i.e. roughly 3:00) of the Bottom Section.

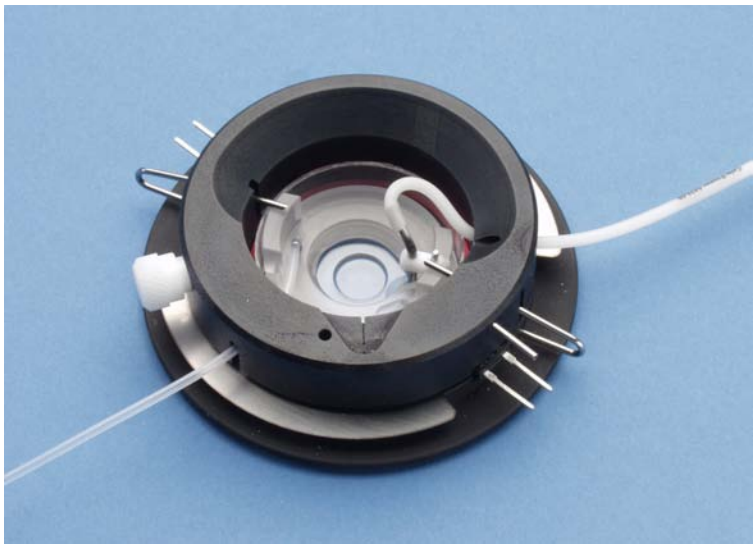
6. Slip a portion of the **PE-50** tubing attached to the perfusion inlet tubing port through its matching slot on the Middle Section (i.e. roughly 7:00).

7. Place the left-side narrow slot and wide slot of the Middle Section on top of the matching left-side clamp and resister respectively (i.e. roughly 9:00) of the Bottom Section.

8. The Middle Section of the **DH-35i** should now be able to mount directly on top of the Bottom Section without any further restrictions.

9. Lift both pieces of flexible tubing from the **RC-37** insert to the top of their respective slots in the Middle Section of the **DH-35i**. The flexible tubing for the perfusion inlet port typically aligns itself in its slot with no difficulty and you can pull out any "slack" from inside the middle portion of the DH-35i.

   The flexible tubing for the suction port typically requires a small degree of "looping" to enable it to both fit in its slot and to prevent kinking of the suction tubing. (See figure to the right). Re-adjust the height of the suction tubing via its Teflon component.



10. Place the Top Section of the **DH-35i** on top of the Middle Section. There is an aligning hole on the top of the Middle Section and a matching pin on the bottom of the Top Section to facilitate proper alignment. There are also three additional pins on the bottom of the Top Section which fit around the outside edges of the Middle Section.

11. If heating with Warner's **TC-324B** or **TC-344B** Temperature Controllers, slip the white colored T1 feedback thermistor on the **CC-28** cable into the milled hole in the side of the Bottom Section. A small amount of mineral oil can facilitate thermal contact between the T1 feedback thermistor and the metal of the Bottom Section. Slip the blued colored leads over the two resistive heating elements and then plug the **CC-28** cable into the temperature controller.

    Consult the **TC-324B/TC-344B** user's manual for methods of controlling the temperature of the clamped culture dish.

12. If imaging, place the fully assembled **DH-35i** into your microscope's **Series 20 Stage Adapter** and mount onto the microscope.

OPERATION

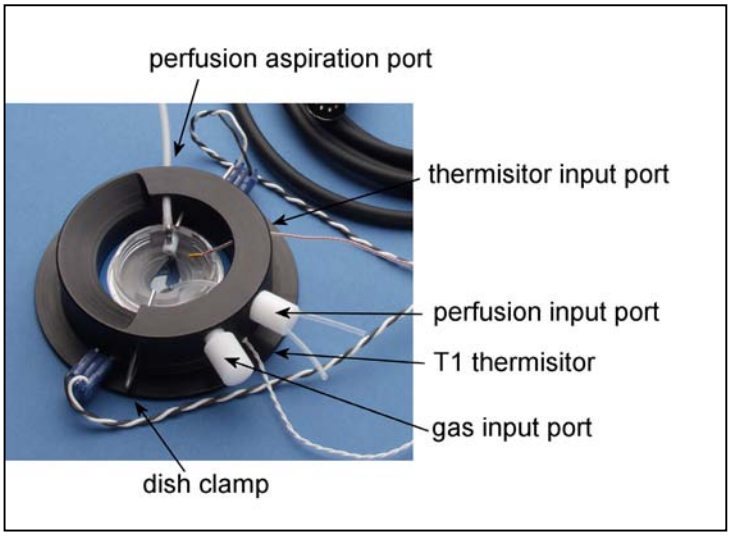
Open Format Applications

Only the Bottom Section is required for open format use, together with one of the four colored adapter rings and one set of dish clamps. The open format enables easy access for microelectrodes, as well as for perfusion and aspiration lines. Cell culture dishes can be used with and without covers, and dish perfusion can be simplified by use of Warner's **RC-37** Perfusion Chamber Insert.

The open format permits imaging using either upright or inverted microscopes. Warner **Series 20** stage adapters are used to mount the **DH-35i** onto most commonly used microscope stages. Contact our Tech Support Department for custom stage adapters.

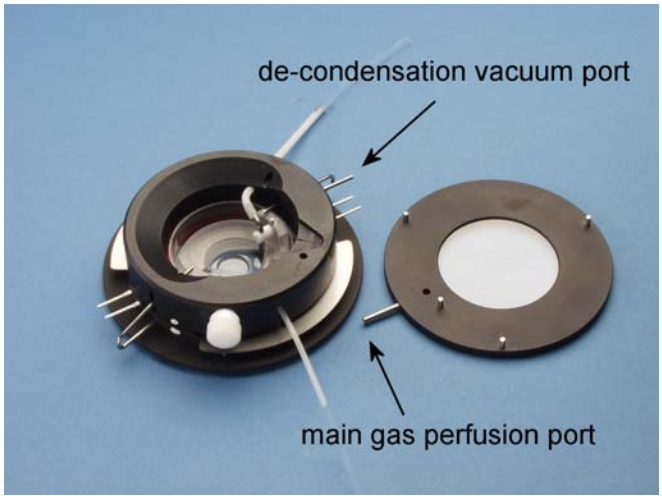
Resistive heating of the **DH-35i** is achieved using Warner's **TC-324B** or **TC-344B** Temperature Controller and a **CC-28** cable. The two blue **CC-28** connectors attach directly to each resistive heating element. Feedback control is provided thermistor (i.e. T1) is inserted in the Bottom Section's metal chassis via a special hole. (see photo) We recommend using a drop of mineral oil to insure good thermal contact between the white T1 thermistor and the metal chassis. The **CC-28** cable's bath thermistor (i.e. T2) can be placed directed in the bath to check solution temperature. The temperature control unit regulates the heating current by sensing the temperature of the incubator via the T1 control thermistor in the **CC-28** cable. Heating of perfusate can be achieved using our **SH-27B** or **SF-28** In-Line solution heaters, which are also controlled via our **TC-324B** and **TC-344B** Temperature Controllers.

Warner Instruments **RC-37** perfusion chamber inserts (e.g. **RC-37W, RC-37F**) are also available for Willco, Corning, and Falcon 35 mm cell culture dishes and can be used together with the **DH-35i**. These incorporate our diamond fluidics design to maintain laminar perfusate flow. Fluid level in the cell culture dish can be controlled via a height adjustable aspirator. Agar bridge wells are built into the **RC-37** perfusion chamber inserts, for signal grounding purposes. The combination of the **RC-37** perfusion chamber inserts with our **DH-35i** micro-incubation heater creates the ability to quickly alter the extracellular environment in 35 mm cell culture dishes, by changing either the perfusate or the bath temperature.



Closed Format Applications

In the closed format the Middle Section and windowed Top Section are used together with the Bottom Section described above to maintain an enclosed environment. Gas inlet and outlet ports are available in the Middle Section for atmospheric control of the cell culture dish's environment. This enables the user to control the cell's



pH, [CO_2], and [O_2]. The Middle Section and windowed Top Section are easily removed for replacement of cell culture dishes during replicate trials. The Middle Section locks onto the Bottom Section holding the cell culture dish via the two magnetic posts. Dish clamps can still be used in the closed format, via the slots provided in the Middle Section, to insure good thermal contact between the cell culture dish and the resistive heating elements. A special hole in the Middle Section permits insertion of out T2 bath thermistor in the enclosed cell culture dish. In the closed format resistive heating can be achieved in the same manner as described above for the open format. The closed format permits imaging using inverted microscopes.

APPENDIX

Cleaning and maintenance

The **DH-35i** is constructed of anodized aluminum. It can be easily cleaned with common laboratory detergents or with ethyl alcohol.

Warranty and service

Service

We recommend that all questions regarding service be referred to our Technical Support Department. Normal business hours are 8:30 AM to 5:00 PM (EST), Monday through Friday.

Our offices are located at 1125 Dixwell Avenue, Hamden, CT 06514.

We can be reached by phone at (800) 599-4203 or (203) 776-0664. Our fax number is (203) 776-1278. E-mail us at support@warneronline.com or through the web at http://www.warneronline.com/contact.html.

Warranty

The **DH-35i** is covered by our Warranty to be free from defects in materials and workmanship for a period of one year from the date of shipment. If a failure occurs within this period, we will either repair or replace the faulty component(s). This warranty does not cover failure or damage caused by physical abuse.

In the event that repairs are necessary, shipping charges to the factory are the customer's responsibility. Return charges will be paid by Warner Instruments.